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BRIEF REPORT

Large B-cell lymphoma with *IRF4* rearrangement: a multi-centric study with focus on potential misleading phenotypes

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Abstract

Large B-cell lymphoma with *IRF4* rearrangement (LBCL-*IRF4*) is a rare lymphoid neoplasm, usually occurring in the pediatric/young-adult age. Despite this, subsets of cases occur in elderly patients and express CD5, possibly entering the differential diagnosis with adult aggressive lymphomas, such as blastoid/pleomorphic mantle cell lymphoma (MCL-B/P). To better characterize the clinical-pathological features and differential diagnosis of LBCL-*IRF4*, we conducted a multi-centric study on 12 cases, focusing on CD5, Cyclin D1, and SOX11 expression. While most cases had typical presentation, adult-to-elderly age at diagnosis and unusual anatomic locations were reported in 3/12 (25.0%) and 2/12 (16.7%) patients, respectively. Histologically, CD5 was positive in 4/12 (33.3%) cases, Cyclin D1 was invariably negative, and SOX11 was weakly/partially expressed in 1/12 (8.3%) case. In conclusion, LBCL-*IRF4* can have unconventional clinical presentations that may challenge its recognition. Although CD5 is frequently expressed, negativity for Cyclin D1 and SOX11 contributes to the differential diagnosis with MCL-B/P.

Keywords Large B-cell lymphoma with *IRF4* rearrangement · Pediatric lymphoma · Mantle cell lymphoma · SOX11 · Cyclin D1

Introduction

Large B-cell lymphoma with *IRF4*-rearrangement (LBCL-*IRF4*) is a rare neoplasm, accounting for < 1% of all lymphoid malignancies [1]. It usually affects pediatric and young-adult patients (mean age at diagnosis: 14 years), with barely equal sex distribution [1, 2]. Typically, LBCL-*IRF4* presents as a localized lesion of Waldeyer's ring (WR), cervical lymph nodes (CLNs), or gastrointestinal tract [3, 4]. Conventional chemotherapy is curative in most cases and the overall prognosis is usually excellent [5].

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Histologically, LBCL-*IRF4* is characterized by a follicular to diffuse proliferation of medium to large size blasts with open chromatin and inconspicuous nucleoli. Centroblastic morphology is also common [6]. The neoplastic cells are strongly positive for panB-cell markers, MUM1 and BCL6, with variable expression of CD10, BCL2, and c-Myc. Positivity for CD5 is documented in about 30–35% of cases [1, 6]. Virtually, all cases harbor *IRF4* rearrangements with no evidence of *MYC* and/or *BCL2* translocations [5]. Cryptic *IRF4* rearrangements are also possible and may be inferred from concurrent *IRF4* mutations and immunoglobulin gene breaks [2, 7].

Although the clinical-pathological features of LBCL-*IRF4* are well established, overlap exists with other entities, including diffuse large B-cell lymphoma, not otherwise specified (DLBCL NOS), pediatric-type follicular lymphoma (PTFL) and grade 3B follicular lymphoma (G3BFL)/follicular large B-cell lymphoma (FLBL) [1, 6]. In our practice, we also encountered a challenging case of CD5-positive/MUM1-positive/Cyclin D1-negative aggressive B-cell lymphoma in a young adult with blastoid morphology, diffuse positivity for SOX11 and weak expression of BCL6. This case prompted the very unusual and poorly investigated differential diagnosis between LBCL-*IRF4* and blastoid/pleomorphic mantle cell lymphoma (MCL-B/P). FISH studies showed negativity for both *IRF4* and *CCND1* rearrangements, eventually favoring a diagnosis of Cyclin D1-negative MCL-B/P. Despite this, the lack of published data on SOX11 and Cyclin D1 in LBCL-*IRF4* and the variable positivity for MUM1, CD10, and BCL6 in MCL [8] may hamper the diagnosis of such difficult and very rare cases.

Thus, to better characterize the clinical-pathological features and differential diagnosis of LBCL-*IRF4*, we conducted a multi-institutional study on 12 clinically annotated cases, focusing on CD5, Cyclin D1, and SOX11 expression.

Materials and methods

This retrospective study considered 12 cases of LBCL-*IRF4*, diagnosed between January 2018 and July 2023 in three referral centers for pediatric and adult hematological disorders in Italy (IRCCS-University Hospital of Bologna: $n=8$; Padua University Hospital, Padua: $n=3$; San Raffaele University Hospital, Milan: $n=1$). In these centers, FISH analyses for *IRF4* rearrangements were performed as routine diagnostic practice in any of the following clinical-pathological settings: (i) MUM1-positive B-cell lymphomas of the head and neck region or gastrointestinal tract in children and/or adolescents; (ii) morphologically aggressive B cell lymphomas with germinal center phenotype and strong/diffuse MUM1 expression, irrespective of age and site. To be included in the study, all cases had to fulfill the following criteria: (i) histological diagnosis of LBCL with FISH-confirmed translocation of *IRF4*; (ii) availability of tissue samples for further immunohistochemical and/or FISH characterization; (iii) availability of clinical data, including sex, age at diagnosis, disease site and stage. Whenever available, therapy and follow-up data were also considered.

All cases were reviewed by two pathologists (MP, ES), who were aware of the original diagnosis, but did not have access to the histological reports. They were asked to confirm/exclude the diagnosis of LBCL-*IRF4* (based on immunohistochemical and FISH results), and to assess the cytological, architectural, and immunohistochemical features of each case. In detail, the following parameters were considered: (i) growth pattern of the lymphoid neoplasm (follicular, diffuse, or mixed follicular and diffuse); (ii) cytological features of the neoplastic population (blastoid, centroblastic, immunoblastic); (iii) presence/absence of necrotic areas; (iv) proliferative index (Ki67 immunostain); (v) basic immunophenotype (CD20, CD3, CD10, Bcl6, MUM1, CD5, BCL2, CD30, c-Myc), using previously established cutoffs for positivity [8]. All cases were also stained for Cyclin D1 and SOX11 in the BOND-MAX (Leica Biosystems, Milan – Italy) and BenchMark ULTRA (Roche Diagnostics, Monza (MI) – Italy) automated immunostainers. Details on primary antibodies used for immunohistochemical analysis are reported in Supplementary Table 1.

The diagnosis of LBCL-*IRF4* was confirmed in all cases with no discrepancies in cytological and immunohistochemical assessment. In one case (case #9), sampling artifacts limited architectural evaluation, but a consensus was reached after a joint session to the microscope.

FISH for *IRF4* rearrangements was performed using the Zyto*Light* SPEC IRF4,DUSP22 break-apart probe (ZytoVysion, Bremerhaven—Germany). *MYC*, *BCL2*, *BCL6*, and *IGH* rearrangements were also tested, using IQFISH break-apart probes for the 8q24, 18q21, 3q27, and 14q32 *locus*, respectively (Agilent Technology, Santa Clara – CA, USA). The study was conducted in accordance with the declaration of Helsinki, following local regulations for clinical research.

Results

Clinical features of LBCL-IRF4 patients

The study cohort included 7 males and 5 females (M:F ratio, 1.4), with median age at diagnosis of 27.5 years (range: 9–72 years). All patients presented with low-stage disease (stage I: 7/12 [58.3%]; stage II: 5/12 [41.7%]) and no systemic symptoms. The head and neck region was most commonly affected (9/12 [75.0%]) with either asymmetric tonsil enlargement (7/12 [58.3%]) or unilateral cervical adenopathy (2/12 [16.7%]). Other presentation sites included the

gastrointestinal tract (right colon, 1/12 [8.3%]) and inguinal lymph nodes (2/12 [16.7%]) (Table 1).

Therapy and follow-up data were available in 10/12 (83.3%) cases. Combination chemotherapy *plus* Rituximab was the treatment of choice in 9/10 (90.0%) cases, including all adult patients (R-CHOP or R-CHOP-like regimens) and 2 out of 3 pediatric patients (AIEOP LNH-97 protocol). In the remaining pediatric case, a watchful waiting approach was chosen following complete surgical excision (Table 1). After a median follow-up of 61.8 months (range: 23.3–140.2 months), all patients, who completed treatment, were alive and in complete remission. One patient (case #12) is currently under therapy.

Histological and cytogenetic features of LBCL-IRF4

On histology, nodal and tonsil architecture was effaced by a lymphoid proliferation of medium to large blasts with follicular (2/12 [16.7%]), diffuse (3/12 [25.0%]), or mixed (follicular and diffuse; 7/12 [58.3%]) growth pattern. In most cases (9/12 [75.0%]), the neoplastic cells had centroblastic morphology with round to vaguely irregular nuclei, coarse chromatin, and evident nucleoli. The remaining 3/12 (25.0%) cases had blastoid features with small-to-medium nuclei, finely dispersed chromatin and inconspicuous nucleoli (Table 2). Necrotic areas were present in 2/12 (16.7%) cases.

Phenotypically, all cases disclosed strong and diffuse positivity for CD20, MUM1, and BCL6. CD10 was expressed in 7/12 (58.3%), BCL2 in 8/12 (66.7%), and c-Myc in 10/12 (83.3%) cases. Partial positivity for CD30 was documented in 3/8 (37.5%) tested cases, while CD5 was detected in 4/12 (33.3%) samples, varying from weak/partial (2 cases) to strong and diffuse (2 cases). The median Ki67 proliferation index was 87.5% (range: 20–98%) (Table 2; Fig. 1).

As per the inclusion criteria, all cases were positive for *IRF4* rearrangements by FISH studies. *BCL2* and *MYC* rearrangements were not detected in any case, while *BCL6* rearrangements were documented in 3/12 (25%) cases. *IGH* rearrangements were present in 10/12 (83.3%) cases (Table 2).

Cyclin D1 and SOX11 expression in LBCL-IRF4

To date, no studies have assessed Cyclin D1 and SOX11 expression in LBCL-*IRF4*. Such information could nevertheless support the differential diagnosis between CD5-positive LBCL-*IRF4* and very rare cases of Cyclin D1-negative MCL-B/P, which may mimic each other both morphologically and phenotypically (i.e., partial overlap of MUM1, CD10, and BCL6 expression patterns) [8, 9].

Moving from these premises, we performed Cyclin D1 and SOX11 immunostains in all of our cases. Overall, 11/12 (91.7%) LBCL-*IRF4* were negative for both Cyclin D1 and SOX11. The remaining case showed weak positivity for SOX11 in about 20% of neoplastic cells, with no evidence of Cyclin D1 expression. Of note, all CD5-positive LBCL-*IRF4* of our series were negative for both Cyclin D1 and SOX11 (Fig. 1).

Discussion

First described in 2011 [2], LBCL-*IRF4* has been formally recognized as a definite entity by the 2022 WHO and International Consensus Classifications of lymphoid tumors [3, 4]. LBCL-*IRF4* typically affects pediatric and young-adult patients, presents as early-stage disease in WR, CLNs or (less commonly) in the gastrointestinal tract, and has very favorable prognosis after conventional

Table 1 Clinical features of LBCL-*IRF4* patients

Pts no	Sex	Age (y)	Disease site	Disease stage	Therapy	FU duration (mo)	Status at last FU
#1	Male	22	Palatine tonsil	IA	6×R-CHOP	61.8	CR
#2	Female	28	Inguinal LN	IIA	n.a	n.a	n.a
#3	Male	38	Palatine tonsil	IA	6×R-CHOP	88.7	CR
#4	Female	72	Palatine tonsil	IIA	4×R-COMP	58.8	CR
#5	Male	27	Palatine tonsil	IA	4×R-CHOP	36.6	CR
#6	Female	63	Cervical LN	IA	n.a	n.a	n.a
#7	Female	10	Inguinal LN	IIA	Surgical excision	28.2	CR
#8	Female	45	Cervical LN	IA	4×R-CHOP	23.3	CR
#9	Male	9	Large bowel (cecum)	IIA	AIEOP-LNH97 protocol	140.2	CR
#10	Male	12	Palatine tonsil	IIA	AIEOP-LNH97 protocol	100.9	CR
#11	Male	23	Palatine tonsil	IA	6×R-CHOP	104.4	CR
#12	Male	30	Palatine tonsil	IA	3×R-CHOP	2	Under treatment

Abbreviations: *CR* complete response, *FU* follow-up, *LN* lymph node, *R-CHOP* Rituximab *plus* cyclophosphamide, doxorubicin, vincristine, and prednisone, *R-COMP* Rituximab *plus* cyclophosphamide, vincristine, liposomal doxorubicin, and prednisone

Table 2 Histological and cytogenetic features of LBCL-*IRF4*

Pts no	Growth pattern	Cytology	Immunohistochemical analysis											FISH analysis				
			CD20	MUM1	CD10	BCL6	BCL2	c-Myc	CD30	CD5	Cyclin D1	SOX11	Ki67 (%)	*IRF4*	*MYC*	*BCL2*	*BCL6*	*IGH*
#1	Diffuse	Cb	+	+	–	+	+	+	w +	w +	–	–	90	+	–	–	–	–
#2	Mixed	Cb	+	+	+	+	–	+	w +	–	–	–	98	+	–	–	–	+
#3	Follicular	Cb	+	+	+	+	+	–	w +	–	–	–	20	+	–	–	+	+
#4	Mixed	Cb	+	+	–	+	–	+	n.a	–	–	–	95	+	–	–		+
#5	Mixed	Cb	+	+	–	+	+	+	n.a	–	–	–	70	+	–	–	+	+
#6	Mixed	Blastoid	+	+	–	+	–	+	n.a	–	–	–	80	+	–	–	+	+
#7	Follicular	Blastoid	+	+	+	+	–	+	–	w +	–	–	80	+	–	–	–	+
#8	Diffuse	Cb	+	+	+	+	+	+	n.a	–	–	–	70	+	–	–	–	+
#9	Mixed	Cb	+	+	–	+	+	+	–	–	–	–	95	+	–	–	–	+
#10	Mixed	Cb	+	+	+	+	+	+	–	+	–	–	85	+	–	–	–	+
#11	Mixed	Cb	+	+	+	+	+	–	–	–	–	w +	90	+	–	–	–	+
#12	Diffuse	Blastoid	+	+	+	+	+	+	–	+	–	–	90	+	–	–	–	–

w + indicates weak positivity in < 25% neoplastic cells. *Abbreviations*: *Cb* centroblastic, *FISH* fluorescence in situ hybridization

chemotherapy. Virtually, all cases are strongly positive for MUM1, express ≥ 1 germinal center marker, and bear *IRF4* rearrangements in the absence of *MYC* and *BCL2* translocations [5].

Here, we report the clinical-pathological and FISH features of a multi-centric series of LBCL-*IRF4*, diagnosed in both pediatric and adult patients. Our aim was to describe this entity in detail, specifically addressing misleading clinical presentations and/or unusual phenotypes. Due to the retrospective design of our study and to the criteria for *IRF4* testing in routine diagnostic practice (see *Materials and Methods* section), we did not aim at providing any esteem of the incidence and/or prevalence of this condition.

While in our series the typical features of LBCL-*IRF4* are largely prevalent, unusual presentations were also documented. In keeping with prior studies [6, 7, 10], we found *bona fide* LBCL-*IRF4* occurring in adult-to-elderly patients (age at diagnosis ≥ 45 years: 3/12 [25.0%] cases) and in unusual anatomic sites (inguinal LN: 2/12 [16.7%] cases). Such heterogeneity has direct practical implications, as it suggests considering LBCL-*IRF4* in the differential diagnosis of low-stage, nodal B-cell lymphomas with centroblast/blastoid morphology and strong MUM1 expression, regardless of patient age and site of presentation. This observation is in keeping with the results of a recent study on DLBCL with aberrant CD10, BCL6, and MUM1 coexpression, which highlighted a subset of adult cases with *IRF4* rearrangement and clinical-pathological features akin to pediatric LBCL-*IRF4* [7].

The differential diagnosis of LBCL-*IRF4* primarily includes PTFL (in young patients), G3BFL/FLBL (in adults), and DLBCL NOS [1, 6]. In cases with pure follicular growth pattern, distinction from follicular lymphoma (both G3BFL/FLBL and PTFL) relies on immunophenotyping (i.e., strong MUM1, variable CD10 expression) but, most importantly, on FISH for *IRF4* [6, 11]. In cases with diffuse or mixed growth pattern, distinction from DLBCL NOS relies on a high degree of suspicion, on clinical-pathological correlations, and on FISH and molecular studies [2, 7, 12]. In this context, *IRF4* rearrangements should be interpreted with caution, to exclude their occurrence as secondary genetic events in other B or T-cell lymphomas [13–15]. This is particularly relevant in cases with concurrent *MYC* and/or *BCL2* translocations, which virtually rule out LBCL-*IRF4* and point toward a diagnosis of DLBCL NOS or related entities [7, 10, 16].

Besides G3BFL/FLBL and DLBCL NOS, adult CD5-positive LBCL-*IRF4* may rarely enter the differential diagnosis with MCL-B/P. Putatively, Cyclin D1 and SOX11 should distinguish between these tumors, but this issue has never been investigated in detail. Even greater uncertainties exist in the differential diagnosis between CD5-positive LBCL-*IRF4* and rare cases of Cyclin D1-negative MCL.

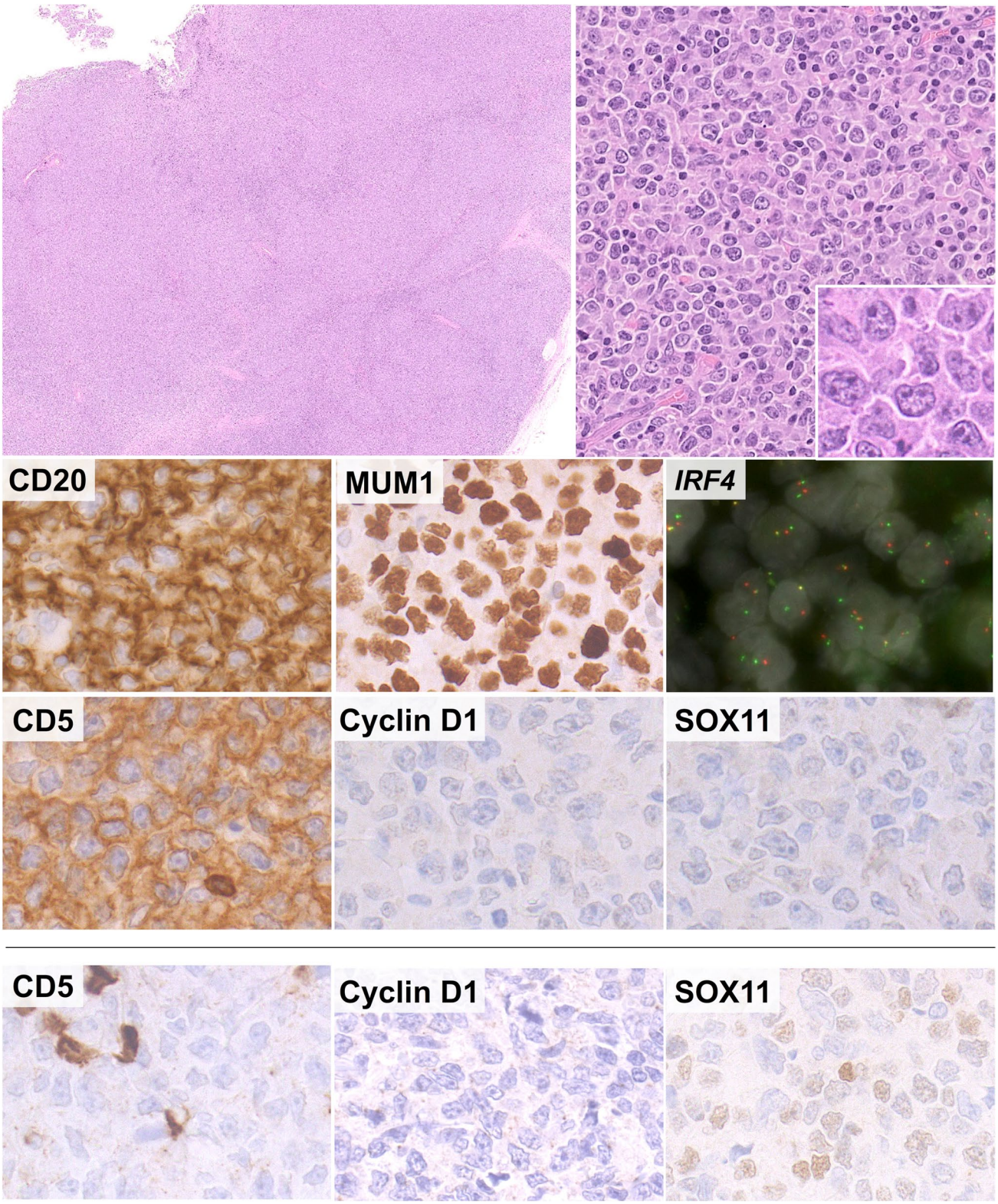

Fig. 1 Representative histological features of LBCL-*IRF4*. Classic morphologic and phenotypic findings of LBCL-*IRF4* are reported in the upper part of the figure (case #10). This case disclosed mixed follicular and diffuse growth pattern with centroblastic cytology. The neoplastic cells were strongly positive for CD20 and MUM1, while FISH analysis documented *IRF4* rearrangement (split signals with ZytoLight SPEC IRF4,DUSP22 break-apart probe). CD5 was positive, while Cyclin D1 and SOX11 were consistently negative. The single SOX11-positive case of this series (case #11—lower part of the figure) was negative for CD5 and Cyclin D1. SOX11 was weakly expressed in only a minority of neoplastic cells. (H&E and immunoperoxidase stain; original magnifications, ×5, ×20, ×40, and ×63)

To address this issue, we assessed Cyclin D1 and SOX11 in all the LBCL-*IRF4* of our series. Cyclin D1 was negative in all cases, while SOX11 was weakly expressed in only 1/12 (8.3%) case. Overall, these results confirm the utility of Cyclin D1 and SOX11 in the differential diagnosis between CD5-positive LBCL-*IRF4* and MCL-B/P, particularly in cases where FISH for *CCND1* and *IRF4* are pending, unavailable or uninformative.

In this context, also, FISH for *BCL6* and *IGH* rearrangements may be of use in the diagnostic workup. In keeping with prior studies [1, 10], we documented *BCL6* rearrangements in 3/12 (25.0%) cases and *IGH* rearrangements in 10/12 (83.3%) cases of our series (Table 2). While *BCL6* translocations are of limited value in the differential diagnosis between LBCL-*IRF4*, FL and DLBCL NOS [2], their occurrence support distinction between LBCL-*IRF4* and MCL-B/P. Likewise, testing for *IGH* rearrangements may support the diagnosis of LBCL-*IRF4* in cases with *IRF4* mutations and cryptic *IRF4* translocations by FISH.

In conclusion, LBCL-*IRF4* is a rare B-cell lymphoma with unique clinical-pathological features. Adult age at diagnosis and/or unusual sites of presentation should not preclude a diagnosis of LBCL-*IRF4*, if all other diagnostic features are present. In particular, FISH for *IRF4* should be strongly considered in all low-stage, morphologically aggressive B-cell lymphomas with strong MUM1 expression and (at least partial) germinal center phenotype, irrespective of patient age and/or anatomic site. In such conditions, *IRF4* rearrangements should always be tested together with *MYC* and *BCL2* rearrangements, as their presence virtually exclude LBCL-*IRF4* [5]. This FISH testing workup has been recently adopted in our diagnostic practice and will hopefully provide more details on the prevalence and clinical features of LBCL-*IRF4*. In adult and elderly patients, Cyclin D1 negativity and inconsistent SOX11 expression support the differential diagnosis with MCL-B/P. Due to the still limited number of reported cases, further studies are needed to confirm our observations and the clinical-pathological features of LBCL-*IRF4*.

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Author contribution MaPiz: writing of the paper and collection of clinical-pathological data; LuBo, FS, CA, LS: collection of histological data and manuscript editing; MaPil, EC, LM, SR, FV: collection of clinical data; SR, PB, LL, LaBo: FISH analysis; MaPo, APDT, ES: project supervision.

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Data Availability Data available from the Authors on request.

Declarations

Conflict of interest The authors declare no competing interests.

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